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ARTICLE

New molecular targets for PET and SPECT imaging in neurodegenerative diseases

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Abstract
The pathophysiology of neurodegenerative diseases (ND) such as Alzheimer's disease (AD) and Parkinson's disease (PD) has not yet been completely elucidated. However, in the past few years, there have been great knowledge advances about intra-and extracellular proteins that may display impaired function or expression in AD, PD and other ND, such as amyloid beta (Aß), α-synuclein, tau protein and neuroinflammatory markers. Recent developments in the imaging techniques of positron emission tomography (PET) and single photon emission computed tomography (SPECT) now allow the non-invasive tracking of such molecular targets of known relevance to ND *in vivo*. This article summarizes recent findings of PET and SPECT studies using these novel methods, and discusses their potential role in the field of drug development for ND as well as future clinical applications in regard to differential diagnosis of ND and monitoring of disease progression.

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Introduction

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are *in vivo* imaging techniques that allow the non-invasive tracking of brain pathophysiological processes underlying various neurological and psychiatric disorders. These techniques have also been successfully used in various aspects of drug development, including the understanding of the mechanism of action of pharmacological agents in the central nervous system (SNC), their dosage regimens and thresholds for clinical response and emergence of side-effects.[1]

Several studies over the past decades have shown that PET and SPECT methods can reliably map neurochemical processes of interest in the brain, including the density and affinity of postsynaptic receptors for neurotransmitters such as dopamine, serotonin and others, as well as presynaptic transporters for these transmitters, precursors such as L-DOPA and transmitter degrading enzymes. Such approach has provided invaluable information about neurochemical abnormalities involved in psychiatric and neurological disorders, as well as helping to elucidate the mechanism of action of the pharmacological agents commonly used to treat these conditions.

More recently, technological advances have enabled the use of PET and SPECT to probe a number of other intra- and extra-cellular proteins that may display impaired function or expression in brain diseases. Such advances have moved the neurological and psychiatric uses of PET and SPECT from a strict neurochemical imaging role to a much more flexible and comprehensive profile of applications, providing knowledge about molecular brain mechanisms that may be much closer to the pathophysiological essence of neurological and psychiatric disorders than superficial neurotransmitter changes.

One of the most promising applications of such new PET and SPECT methods regards to the investigation of pathophysiological aspects of neurodegenerative disorders (NDs). This is of great relevance given the large prevalence of NDs such as Alzheimer's disease (AD) and Parkinson's disease (PD) in elderly life, as well as the fact that a greater knowledge about the pathophysiology of these disorders may help in the development of novel pharmacological treatments capable of interfering with their molecular pathological substrate. Taken those issues into account, this review will focus on perspectives for new PET and SPECT tracers developed to allow the mapping of intracellular and extracellular mechanisms of particular relevance to AD, PD and other NDs.

Molecular brain imaging with PET and SPECT: basic principles

In order to allow the efficient visualization, characterization and quantitative measurements of relevant biological processes in the brain, PET and SPECT techniques demand the development of suitable probes that can be labeled with a positron emitting isotope (in the case of PET) or photon emitting isotope (in the case of SPECT). Importantly, because of their limited spatial resolution, the use of computed tomography (CT) or magnetic resonance imaging (MRI) is often required. Functional and structural techniques can be easily fused using special software, by creating parametric images. However, the development of hybrid systems where functional techniques are fully integrated with structural cross-sectional methods also helped to attenuate the lack of anatomical resolution of PET and SPECT. These parametric images give both anatomical and functional information, allowing the identification of regions which exhibit differences in the uptake of labeled compounds. Anyway, the most employed radioisotopes for labeling PET probes are carbon-11 (^{11}C) and fluorine-18 (^{18}F), differing basically in their half-lives and maximum energy. The first (^{11}C) must be produced by an on-site cyclotron located near to the PET imaging facility due to the very short physical half-life (20 minutes). However, the longer half-life of ^{18}F (110 minutes) allows the delivery of ^{18}F-labeled ligands to a broader list of PET facilities located in the same town, or even in neighborhood cities. For SPECT imaging, probes can be labeled with iodine-123 (^{123}I) or technetium-99m (^{99}mTc);[2] these isotopes have much longer half-lives than those used in PET imaging, avoiding the need for a nearby cyclotron.

Having crossed the blood-brain barrier (BBB) after intravenous injection, the radiolabeled compound accumulates in certain parts of the brain, depending on the biological process being tracked. Both PET and SPECT are equipped with distinct radiation detectors that are placed in close proximity to the head after injection of the radioligand, and the data collected by such detectors are transformed to generate three-dimensional tomographic maps displaying the regional distribution of radioactivity emitted by the brain. In order to be suitable for *in vivo* brain imaging with PET or SPECT, a radiopharmaceutical compound (also called radiotracer, due to its sub-pharmacological dose) needs to be able to bind specifically to its target (binding potential of a drug) (Figure 1), otherwise the accuracy of the imaging information obtained may be impaired. By definition, binding potential (BP) is a pivotal measure in the use of PET to

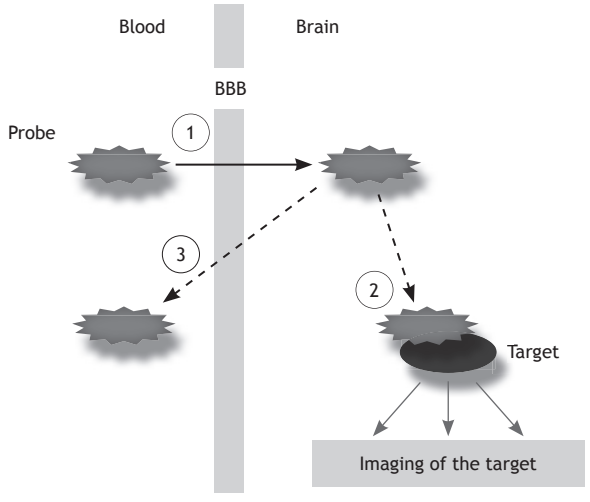

Figure 1 The basic requirements for suitable target-imaging agents include: (1) prompt crossing of the blood-brain barrier; (2) selective binding to the target molecules; and (3) clear and contrasting signals between target and non-target molecules.[4]

measure the density of "available" receptors, e.g. to assess the occupancy by drugs or to characterize abnormalities in receptor distribution in association with neuropsychiatric disorders. Thus, BP is a combined measure that depends on receptor density as well as on drug-receptor affinity.[3]

Amyloid imaging tracers

Extracellular senile plaques are protein aggregates formed by the misbalance between the production and clearance of proteins or peptides in brain tissue of AD patients. Amyloid beta (Aß), released from the cleavage of the amyloid precursor protein (APP), is the most important constituent present in these plaques and represents the main hallmark that characterizes the neuropathological diagnosis of AD. The cleavage of APP can be performed by several proteases or peptidase proteins. Among these, secretases, especially gamma (which contains presenilins, nicastrin, anterior pharynx defective-1, and presenilin enhancer-2) and beta (ß-site APP cleaving enzyme 1, BACE1), are the most important enzymes, with their activity being responsible for the excessive release of the highly amyloidogenic 42 amino acid variant (Aß42) peptide. In contrast, APP cleavage promoted by the α-type secretases disintegrin and metalloproteinase (ADAM) 10 and 17 contributes to the formation of soluble neuroprotective fragments known as Sα-APP.[5]

The great advances in the knowledge about the molecular basis of AD, described above, have led to enormous interest in the development of PET and SPECT tracers that could be useful for *in vivo* imaging of Aß plaques in the human brain. The first PET tracer developed to bind specifically to fibrillar Aß plaques was the ^{11}C-labeled Pittsburgh Compound-B ([^{11}C]PIB). Up until now, this has been the best characterized and most widely used PET tracer for the study of amyloid deposits in the human brain, both in AD and in other NDs. The several potential roles of *in vivo* amyloid imaging techniques in AD are outlined in Table 1.

Since a definite diagnosis of AD can only be confirmed by post-mortem neuropathological examination, diagnostic tools that can be used to give support to a suspicion of AD in an individual with memory deficits and other features of cognitive decline are highly valuable. Several studies have shown a marked degree of [^{11}C]PIB retention in the association cortex of mild AD patients compared to healthy controls[6-9] (Figure 2).

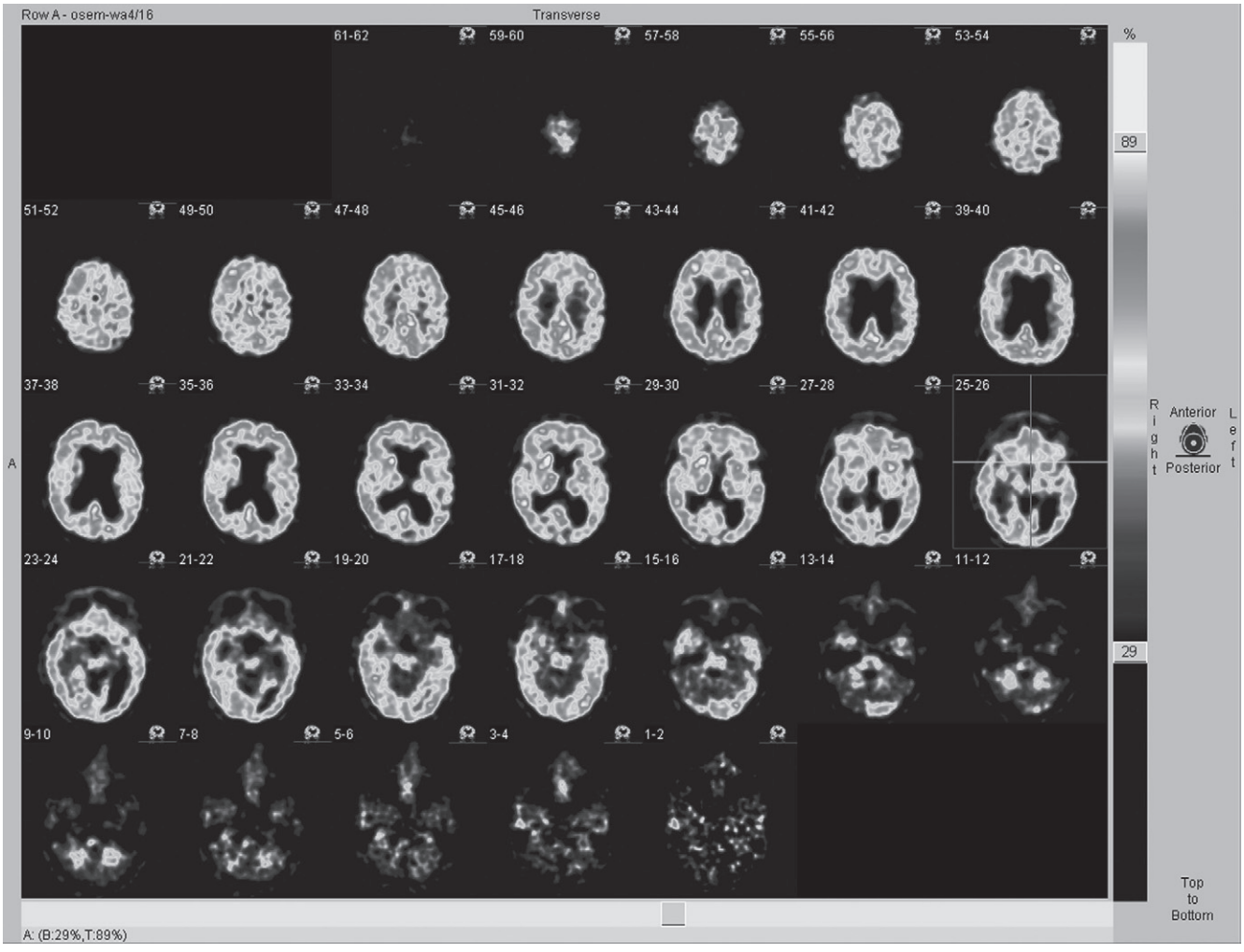

Figure 2 PET images obtained after intravenous injection of 11C-labeled Pittsburgh Compound B (PiB) in a patient with probable Alzheimer's disease, revealing amyloid deposition in the brain. Warmer colors (e.g. red and yellow) indicate greater concentrations of amyloid deposits, while the blue color indicates the absence of these deposits.

Table 1 Uses of amyloid imaging with PET in neurodegenerative disorders

Research applications
• Elucidation of pathophysiological aspects of Alzheimer's disease (AD), minor cognitive impairment and other disorders that involve amyloid deposition in the brain
• Mapping of the progression of brain pathological changes over time
• Evaluation of disease-modifying properties of novel treatments
Potential clinical applications
• Ruling out of Alzheimer's disease in cases of suspected cognitive decline
• Differential diagnosis between Alzheimer's disease and frontotemporal dementia
• Differential diagnosis between dementia with Lewy bodies and Parkinson's disease

These findings established PET imaging with [^{11}C]PIB as a useful imaging tool to aid in the diagnostic confirmation of early AD.[9,10] However, it should be noted that amyloid deposition is not pathognomonic of AD, being for instance found in a proportion of cognitively healthy elderlies. Nevertheless, a negative [^{11}C]PIB PET result is highly informative to rule out the diagnosis of AD.

The usefulness of [^{11}C]PIB imaging with PET for assessing the progression of AD has not been as well established. For instance, an interesting 2-year follow-up study of AD patients revealed that there was no significant increase in [^{11}C]PIB uptake over time, although individually some patients showed clear increases.[8] Such pattern of results indicates Aß deposition plateaus when clinical dementia is already established. PET investigations with [^{11}C]PIB are also clinically useful to aid in the distinction between AD and other dementias. Most notably, patients with frontotemporal dementia (FTD) have generally normal [^{11}C]PIB uptake (although occasional FTD patients may display increased brain uptake).[11,12]

Individuals with objective cognitive decline not severe enough to fulfill the criteria for established dementia receive the diagnosis of minor cognitive impairment (MCI).[13] Subjects diagnosed as suffering from MCI have a high risk of developing dementia, with an estimated rate of conversion to AD of approximately 12% per year.[13] A number of PET studies have shown that a subpopulation of subjects with MCI shows increased levels of [^{11}C]PIB uptake to the same degree as seen in patients with AD.[7,14,15] In addition, recent investigations demonstrate that increased [^{11}C]PIB uptake in the brain of MCI patients is highly predictive of subsequent conversion to AD.[16]

Also noteworthy, most patients with dementia with Lewy bodies (DLB) demonstrate increased [^{11}C]PIB uptake in the brain.[17] Recent reports have shown that [^{11}C]PIB holds promise to help in the discrimination of DLB patients from those with PD, PD with dementia (PDD), PD with mild cognitive impairment (PD-MCI), and healthy control subjects (HCS).[17,18] However, [^{11}C]PIB retention did not differ across the diagnoses of PDD, PD-MCI, PD, and HCS.[18] Importantly, one study reported that the increased [^{11}C]PIB retention in the brains of DLB patients is largely attributable to the binding of [^{11}C]PIB to Aß plaques and not to α-synuclein, the primary structural component of Lewy body fibrils.[19]

Finally, two other important areas of potential use for amyloid imaging tracers include drug development and monitoring of treatment effects (Table 1). For example, one study of AD patients treated with phenserine, an anticholinesterase compound, showed an improvement in cognition that was not however accompanied by significant changes in mean cortical [^{11}C]PIB retention in the brain.[20]

Given the longer half-life of 18F-labeled probes in comparison to [^{11}C]labeled compounds (110 minutes vs. 20 minutes), there has been a great degree of interest in the past few years in the development of ^{18}F-labeled compounds for brain amyloid imaging with PET, which could be transported from radiopharmaceutical production facilities to other PET imaging sites. A number of such 18F-labeled amyloid imaging tracers, including [^{18}F]flutemetamol (an ^{18}F-labeled derivative of PIB), [^{18}F]AV-45 (florbetapir) and [^{18}F]BAY 94-9172 (florbetaben), are already in an advanced stage of clinical trials.[21-24] Noteworthy, florbetapir has been recently approved by the FDA in the United States for PET imaging of the brain in adults under evaluation for AD and other causes of cognitive decline.[25] Clinical studies indicate that these 18F-labeled amyloid imaging tracers provide good discrimination between AD patients and healthy subjects. However, they still present some technical limitations, including a relatively low degree of specific binding *in vivo*, as well as a high level of white matter binding in healthy human brains which reduces the contrast between cortical and non-cortical specific uptake of the tracer.

Furthermore, two technetium (^{99m}Tc) and rhenium (Re) labeled ligands have been recently synthesized for Aß imaging with SPECT. They are derived from the compounds (2-(1-(6-(dialkylamino)naphthalen-2-yl)ethylidene)malononitrile (DDNP) and 1-(6-(dialkylamino)naphthalen-2-yl) ethanone (ENE). However, these compounds showed low affinities for Aß aggregates and require further refinements in order to improve their diffusion through the BBB.[26] In addition, it is also important to mention that SPECT has lower sensitivity and spatial resolution while compared to PET, and this might also promote possible differences in accuracy between these two techniques.

Tau imaging tracers

In addition to the extracellular Aß plaques mentioned above, intracellular neurofibrillary tangles (NFTs), composed of filaments of microtubule-binding hyper-phosphorylated protein tau, are also an important hallmark of neurodegenerative disorders including AD, being preferentially located in the hippocampus and associative cortical regions.[27,28] Previous neuropathological research suggests that the deposition of NFTs occurs before the manifestation of clinical symptoms in AD and is sufficient to provide a neuropathological diagnosis of AD.[29-31] Thus, *in vivo* imaging of NFTs in conjunction with imaging of Aß plaques would be useful for the early and accurate diagnosis of AD. A quantitative evaluation of tau pathology may also be helpful in tracking the severity of dementia, since the degree of deposition of NFTs correlates well with the clinical severity of dementia. Finally, given that some forms of frontotemporal lobar degeneration are characterized by pathological accumulation of tau protein, tau imaging tracers show also great promise for the diagnosis

of such conditions and their differentiation from AD and psychiatric disorders.[32]

The first radiotracer developed for tau protein imaging in the brain with PET was ^{18}F-FDDNP,[33,34] and this was followed by ^{18}F-FSB[35] and ^{18}F-FP-curcumin.[36] However, all these radioligands bind not only to NFTs but also Aß plaques in the brain.[35,36,37] Therefore, these tracers have limited value for accurately investigating tau-related aspects of AD, or to reinforce the diagnosis of FTD. One additional limitation of [^{18}F]FDDNP is its low signal/noise ratios for PET imaging, due to its reduced specific binding signal and rapid brain uptake of lipophilic metabolites.[37,38]

Recently, however, a series of quinolone derivatives that bind to tau NFTs with higher affinity than ß-amyloid fibrils have been identified.[39] One of these derivatives, 2-(4-aminophenyl)-6-(2-([^{18}F]fluoroethoxy)) quinolone ([^{18}F] THK523), has been evaluated for imaging of tau pathology in the brain with PET.[40] It demonstrated high affinity and selectivity for tau fibrils *in vitro* and *in vivo*. Interestingly, this tracer presented low binding in the brains of transgenic mice overexpressing APP with significant accumulation of cerebral Aß, thus demonstrating its selectivity for tau.[40] Furthermore, auto-radiographic and histofluorescence analyses of human hippocampal serial sections from AD patients exhibited positive THK523 binding that co-localized with immunoreactive tau pathology, while not highlighting Aß plaques. These experiments indicate that [^{18}F]THK523 fulfills the criteria for a proper radioligand that could be used in human imaging trials.

More recently, *in vitro* and *in vivo* studies have also shown that TH2, a novel radioiodinated rhodanine and thiohydantoin (TH) derivative, binds specifically to NFTs and may be suitable for SPECT imaging of tau pathology.[41] One other potential SPECT tracer is the phenyldiazenyl benzothiazole (PDB) derivative 4-[2-(5-methoxy-2-benzothiazolyl) diazenyl]-N, N-dimethyl-benzenamine, which binds to tau aggregates with a two-fold selectivity relative to Aß aggregates.[42] However, biodistribution experiments using normal mice show that PDB derivatives display persistent levels of radioactivity in the brain. This makes them unsuitable for imaging NFTs *in vivo* in humans at the present time, and structural changes to the PDB scaffold may be needed to make these compounds useful for imaging NFTs in the human brain with SPECT.

Lewy Body tracers

One other important area of research refers to the development of PET and SPECT radiotracers capable of binding specifically to Lewy bodies. Such compounds may be highly useful for the diagnosis and assessment of therapy and severity of pathological progression of α-synuclein-associated disorders. α-synuclein is the main constituent of Lewy bodies and is known to interact with several proteins also involved in neurodegeneration.[43] Its pathological accumulation may alter mitochondrial function,[44] synaptic rearrangement,[45] microtubule associated-protein like tau function (because it can interact with tubulin),[46,47] neuronal Golgi apparatus behavior and vesicle trafficking,[48] and cell membrane lipid composition and fluidity.[49]

The compound BF227, initially designed as an Aß imaging agent,[50] was recently demonstrated to label to both Aß plaques and Lewy bodies in immunohistochemical/fluorescence analyses of human brain sections of sufferers of AD and PD, respectively. Thus, [^{18}F]BF2[27] is regarded as a potential non-Aß-selective biomarker for the study of PD.[51] It should be noted that BF2[27] has been recently shown to stain α-synuclein-containing glial cytoplasmic inclusions in post-mortem tissue. In the same report, PET examinations with carbon-11-labelled BF227 ([^{11}C]BF227) detected α-synuclein deposits in the living brains of patients with multiple system atrophy (MSA).[52] This indicates that [^{11}C]BF227 could be a potential tool to monitor the effectiveness of neuroprotective therapy for α-synucleinopathies. However, further studies are warranted to verify whether Lewy bodies in other α-synucleinopathies as well as glial cytoplasmic inclusions can be detected by [^{11}C]BF227 PET.

Tracers for neuroinflammation

Neuroinflammation is a known ageing-related multifactorial process which is commonly found in earlier stages of NDs, and is directly implicated in the progression of these diseases.[53] Up until now, the most widely used tracer to visualize neuroinflammation in the brain has been [^{11}C]PK11195, which is capable of mapping microglial activation through binding of the 18-kDa translocator protein (TSPO), formerly known as peripheral benzodiazepine receptor (PBR). TSPO is mainly found in the outer mitochondrial membrane and is primarily involved in cholesterol transport for further steroidogenesis. In brain tissue, TSPO expression is relatively low. However, a dramatically up-regulation occurs when microglia is activated, which confers to this protein an important role as a neuroinflammatory biomarker in the brain.[54] [^{11}C]PK11195 has been recently used in several studies of psychiatric disorders, revealing patterns of widespread microglia activation in the brain (Figure 3). In NDs, studies with this tracer have shown that microglial activation is indeed an early pathological event,[55-58] thus providing support to the possible use of anti-inflammatory based therapeutic interventions for NDs. However, it is important to consider that it is also in the early stages of NDs that microglia have protective effects, for example, by promoting amyloid clearance. However, these cells become increasingly dysfunctional at later stages, then contributing to disease progression.[59]

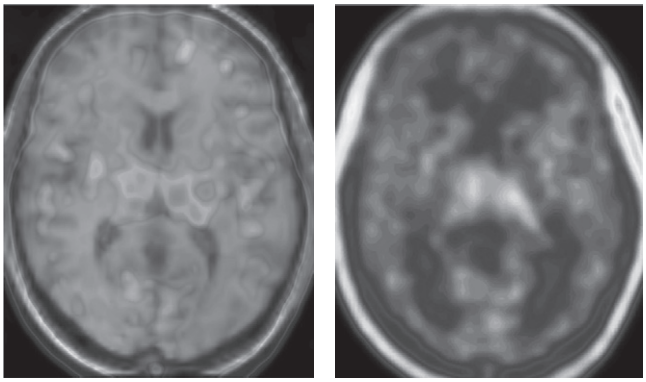

Figure 3 Representative PET parametric image of [^{11}C] PK11195 binding potential (BP) in a healthy individual, superimposed on an MRI (magnetic resonance imaging) template (A); and a [^{11}C]PK11195 PET image from a patient with schizophrenia (B). Note the higher radiotracer uptake in the subject with schizophrenia (in blue color), suggesting the occurrence of neuroinflammatory processes.

Unfortunately, critical issues have limited the use of [^{11}C]PK11195 for measuring neuroinflammation in the brain, including poor bioavailability in brain tissue and high levels of nonspecific binding.[60] That high level of nonspecific activity makes the interpretation of images very complex and cumbersome. More recently, several other TSPO-related PET tracers have been characterized and are being used in preclinical and clinical studies of neuroinflammation in association with NDs (see Ching et al.,[61] for review). Such radiotracers, including [^{11}C]DPA-713 and the 2-Phenylimidazo[1,2-a]pyridineacetamide derivative, [^{11}C]CLINME, may be more suitable for visualizing mild neuroinflammation than [^{11}C]-(R)-PK11195, given that they: are more sensitive for the detection of small amounts of TSPO; have lower levels of non-specific binding; and provide higher signal to-noise ratios. Such properties have been evaluated using infection models, whereby the rate of tracer uptake in infected areas is compared to the uptake in healthy tissues.[62,63] Such superior properties in comparison to [^{11}C]-(R)-PK11195 have also been demonstrated for [^{18}F]PBR111, the fluorinated version of [^{11}C]CLINME,[64] and [^{18}F]DPA-714,[62] with the advantage that these latter tracers are labeled with 18F (110-minute half-life). Noteworthy, recent preclinical TSPO imaging studies have been successfully conducted using models of multiple sclerosis and glioma with [^{18}F]DPA-714.[65,66,67]

Several other new potential molecular targets for neuroinflammation have emerged recently. The activity of ß-glucuronidase, a lysosomal enzyme that is released from reactive astrocytes and microglia, has been successfully imaged recently in a HSV-1-induced encephalitis rat model using 18F-FEAnGA68 (Figure 4). Also, the imidazoline2 Binding Site (I_2BS), I(2)R, has been shown to be altered in several brain disorders including ND.[69] Several ligands for I_2BS, including deprenyl, are able to inhibit monoamine oxidase (MAO),[70] whose activity is increased in AD human brain astrocytosis (or astrogliosis, i.e. an abnormal increase in the number of astrocytes due to neurotoxicity or brain injury) measured by [^{11}C]DED[71] (see below). Thus, imaging I_2BS is a promising tool for the study of neuroinflammation in NDs. Recently, the ligand [^{11}C]FTIMD was evaluated with an improved ultra-high specific activity which afforded the detection of small changes in I(2)R expression in the rat brain.[72] Another potentially useful ligand is BU99008, which demonstrates better *in vivo* brain uptake and specificity in comparison with [^{11}C]FTIMD.[73]

Finally, cyclooxigenase (COX) enzymes are widely known as key molecular targets for anti-inflammatory drugs. Recently, [^{11}C]ketoprofen methyl ester was pre-clinically evaluated and proved to be efficient in quantifying COX-1 expression in both neuroinflammation and neurodegeneration models. In addition, it afforded better results than [^{11}C]PK11195 in quantifying neuroinflammation. Therefore, [^{11}C]ketoprofen methyl ester demonstrated to be sensitive for neuroinflammatory processes targeting COX-1 in activated microglia and macrophages.[74]

Moreover, as astrocytosis is a commonly observed phenomenon involved in neuroinflammation and neurodegeneration, this may also be evaluated in the human brain using PET tracers. The most relevant radioligand evaluated for this purpose to date is 11C-deuterium- L-deprenyl or [^{11}C]DED, as mentioned before. Recent studies demonstrated an increase in [^{11}C]DED binding throughout the brain of AD patients that also display high levels of [^{11}C]PIB uptake, suggesting that astrocytosis is an early phenomenon in the development of AD, probably being an intermediate step between amyloidosis and neuronal loss.[75,76]

Finally, there are also promising results in the use of SPECT tracers for neuroinflammation. For instance, [^{123}I]PK11195 has recently been used in a pilot SPECT study with AD patients.[77] In addition, the ^{123}I-radiolabeled compound 6-chloro-2-(4'iodophenyl)-3-(N,N-diethyl)-imidazo[1,2-a]pyridine-3-acetamide or [^{123}I]CLINDE was also successfully tested in preclinical studies. Using different animal models of neuroinflammation, [^{123}I]CLINDE demonstrated good performance to assess TSPO changes related to both astroglial and microglial activation.[78,79] There are also preliminary investigations of [^{125}I]DPA-713 in rats exposed to a seizure-inducing neurotoxicant; these studies revealed increased brain radioactivity in neurotoxicant-treated rats compared with controls, which was completely blocked by administration of PK11195.[80]

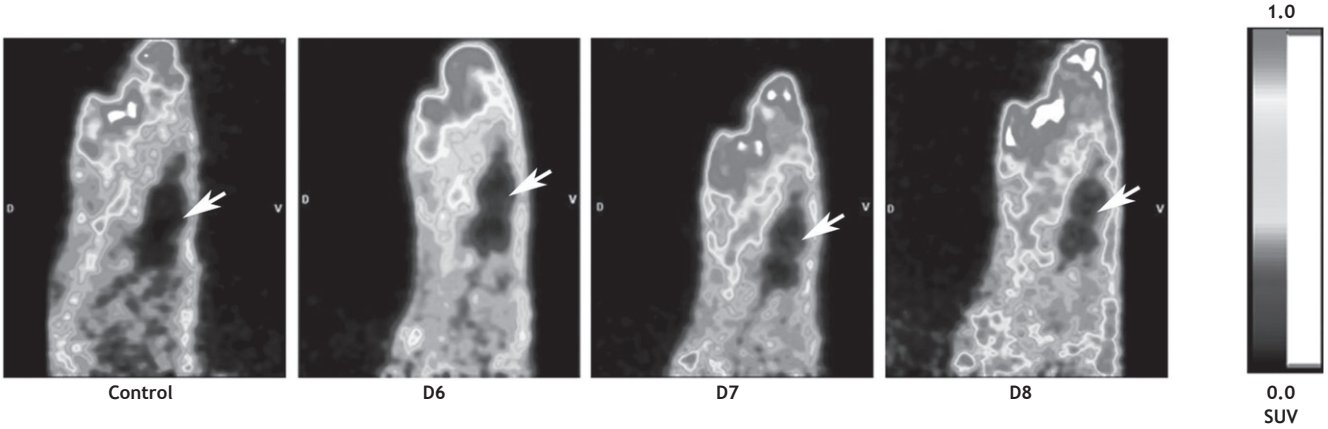

Figure 4 Sagittal view of the head of a control rat (control) and a rat infected with HSV-1 (day 6(D6); day 7 (D7) and day 8 (D8) after virus inoculation). The images represent tracer uptake between 10 and 60 minutes after injection of [^{18}F]FEAnGA. Note the time-dependent microglial activation in the brain (arrows).

Tracers for brain lipid metabolism

Arachidonic acid (AA) and docosahexaenoic acid (DHA), an omega-6 and omega-3 polyunsaturated fatty acid (PUFA), respectively, are very important constituents of phospholipids in cell membranes and contribute extensively to cell signaling in the brain. AA can be obtained from the conversion of its precursor linoleic acid obtained from the diet, whereas the brain concentration of DHA depends on dietary DHA content as well as liver synthesis from its precursor, α-linolenic acid.[81,82] The CNS response to injury and to the onset (and progression) of neurodegeneration involves the release of free DHA and AA along with the synthesis of stereospecific docosanoid derivatives and prostanoids, respectively.[82,83] The release of AA in such conditions is mediated by specific phospholipases, e.g. PLA2, which contribute to the conversion of AA into inflammatory molecules such as prostaglandin E2 (PGE2) by the cyclooxygenase (COX) 1 and 2 enzymes. Interestingly, a recent study trying to understand the role of PLA2 in NDs demonstrated that the inhibition of PLA2 in rat brain leads to a decrease in total tau protein.[84] On the other hand, DHA has anti-inflammatory properties and their docosanoid derivative (e.g. neuroprotectin D1) displays a neuroprotective bioactivity in the brain against various insults, including oxidative injury, ischemia-reperfusion, and inflammation. In addition, low concentrations in the brain have been detected in patients with brain disorders such as AD and depression.[83,85,86,87] Importantly, measured rates of AA and DHA incorporation into brain phospholipids represent their respective rates of metabolic consumption, because these PUFAs are not synthesized de novo or converted significantly from their precursors in the brain[82] (see below).

In recent PET investigations, an increase of 26% in the global brain incorporation of AA in AD patients compared with healthy subjects was observed using [^{11}C]arachidonic acid ([^{11}C]AA).[88] Such incorporation was particularly increased in brain regions where neuroinflammation is thought to be present in AD, and [^{11}C] AA could thus be a novel marker of activated microglia to be used in studies of neurodegenerative disorders. Further studies have evaluated the positron-labeled [1-(11)C]DHA tracer to map the incorporation of unesterified plasma DHA into the brain of healthy adult human volunteers. Values of incorporation coefficients k* for DHA were higher in gray than white matter brain regions. For the entire human brain, the net DHA incorporation rate, J_{in}, the product of k* and the unesterified plasma DHA concentration, equaled 3.8 ± 1.7 mg/day. The authors highlighted that this net rate, approximating the net rate of DHA consumption by brain, is less than the recommended human dietary DHA supplementation of 200 mg per day.[89] Thus, with the use of [1-(11)C]DHA, it is possible to quantify regional and global human brain DHA metabolism in relation to health and disease.[90] In addition, a more recent study demonstrated that is possible to measure brain incorporation of plasma DHA *in vivo*. Thus, quantitative imaging of DHA incorporation from plasma into the brain can be used as an *in vivo* biomarker of brain DHA metabolism and neurotransmission.[87] Importantly, this may help to monitor DHA consumption *in vivo* in patients with disorders such as depression and AD, in which DHA supplementation may be helpful.[91-93]

Other new molecular targets for neuroimaging in neurodegeneration

A potent and selective protein kinase C (PKC) inhibitor, Enzastaurin (LY317615), was recently labeled with 11C for PET imaging applications ([^{11}C]Enzastaurin).[94] PKC is an enzyme involved in several cell biology mechanisms and is one of the most important initial elements involved in the induction of the previously mentioned α-secretases, ADAM-10 and 17, which are involved in neuroprotection.[95] Also, a sensitive myelin probe, [^{11}C]MeDAS, was recently synthesized and proved to be effective in detecting myelin changes in the brain. This radiotracer, which can be used as a myelin-imaging marker to early monitor myelin degeneration *in vivo*,[96] is a potentially useful development for the investigation of NDs, since degeneration of neurons, axons, and synapses is clearly present in AD as much as in multiple sclerosis.[97]

Heat shock proteins (HSP) also display important roles in neuroprotection. HSP70, for example, is a known protein found in aggresome of Lewy bodies and is mainly implicated in the degradation of aberrant proteins.[98] Aggresome is a general response of cells which occurs when the capacity of the proteasome (involved in degradation of unusable proteins) is exceeded by the production of aggregation-prone misfolded proteins.[99] Similarly, cathepsins are also critical for the degradation of enzymes that may be implicated in NDs.[100] Thus, impaired function of these proteins may facilitate the progression of NDs. Interestingly, a PET reporter system (i.e. which uses reporter genes) for imaging gene expression in the intact brain was recently used to image and monitor the activation of the heat shock factor 1 (HSF1)/HSP70 transcription factor.[101] In addition, another group recently imaged the activity of the cysteine cathepsin using ^{64}Cu-Z-FK(DOTA)-AOMK and PET. Imaging of these proteins using the more widely available ^{18}F radioisotope tracer may provide a great tool in the future for early diagnosis and monitoring of disease progression of NDs.

Innate immune responses also play an important role in neurodegeneration. For example, it was recently found that monocytes and microglia that are deficient for myeloid differentiation factor 88 (MyD88) (involved in Toll-like receptor signaling pathway) exhibit a functionally impaired phagocytic reaction to Aβ.[102] In addition, MyD88 is involved in the dopaminergic neuronal degeneration induced by the neurotoxin MPTP in the enteric nervous system (ENS) of the mouse.[103] However, this neurodegeneration is not a MyD88-dependent mechanism.[104] Thus, more knowledge is needed before PET/SPECT imaging studies can be considered for this new target protein.

Oxidative stress (OS) leading to mitochondrial damage is a major and early phenomenon triggering neurodegeneration.[105,106] Interestingly, a tracer named [^{62}Cu]ATSM, initially designed for the study of tumor hypoxia,[107] was recently used, for the first time, to assess OS in PD. This study demonstrated that striatal OS was enhanced in PD patients compared with controls and increased with the progression of disease severity, particularly in the contralateral striatum.[108] It was further demonstrated that this tracer is very specific for the cells with mitochondrial dysfunction, even under normoxia, thus suggesting that [^{62}Cu]ATSM may indeed be an interesting

tracer for the study of brain disorders involving mitochondrial dysfunction, such as AD and PD.[109]

P-glycoprotein (P-gp) is a known BBB active efflux transporter involved in neuroprotection. Its dysfunction is considered one of the causes of the onset of PD[110,111] and AD.[112] In addition, a correlation between aging and decreased function of this transporter has also been established *in vivo*.[113] Thus, developing methods for imaging P-gp in such diseases is an important challenge nowadays. A number of studies have already been carried out using the radiolabelled P-gp substrate [^{11}C]verapamil in PET studies. However, polar radiolabelled metabolites are formed after injection of this radioligand, and this may result in a non-P-gp-mediated signal as a confounding factor.[114] Therefore, new P-gp tracers for imaging P-gP function in the BBB are needed.

Concluding remarks

The studies reviewed in this article demonstrate the many opportunities to be explored using the already available molecular imaging tracers that map targets of known relevance to NDs, including Aß, α-synuclein, tau protein, and neuroinflammatory markers.

On the other hand, the brain mechanisms underlying NDs have not yet been fully elucidated and other targets of potential relevance to NDs emerge continuously.[115] It is clear the need to develop and use novel molecular imaging compounds for such targets, in addition to those related to Aß, α-synuclein and tau protein, in order to achieve a more complete knowledge about the molecular basis of AD, PD and other NDs. Using such novel molecular imaging compounds, it is expected that PET and SPECT methods will help us to further understand the underlying pathological processes and specific molecular alterations that unfold during early stages of NDs.

With an increasingly larger number of animal research facilities worldwide with access to micro-PET and SPECT technology for preclinical studies, it is expected that pharmacology studies using novel radiotracers will help to identify and validate molecular processes as novel biomarkers to be used as therapeutic targets for treatment, and assess how new drugs are able to modify these biomarkers in animal models of NDs. In this field, one of the most promising strategies should be the use of multi-tracer protocols for the simultaneous evaluation of different molecular targets of relevance to ND. For instance, recent investigations using transgenic mice that express pathologies that characterize both dementia with Lewy bodies (DLB) and AD (DLB-AD mice) have revealed that Aß, tau, and α-synuclein act synergistically to promote the aggregation, phosphorylation, and accumulation of each other, as well as leading to accelerated cognitive decline.[116] Thus, multi-tracer protocols for these three molecular targets must be strongly considered in investigations of NDs.

Finally, it would be highly desirable to translate, in the forthcoming years, some of the above novel findings into direct and objective diagnostic applications in clinical practice. With such developments, PET and SPECT imaging patterns might be used more incisively to improve diagnostic accuracy in doubtful cases, as well as to predict prognoses and treatment response in sufferers of NDs. The larger availability of SPECT and its lower costs may make it the method of choice for such future clinical applications, but a progressively greater access to PET methods across a larger number of hospitals is also expected, particularly in regard to the use of ^{18}F-labeled tracers. The use of any novel radiotracer for clinical applications with PET or SPECT should be weighted against the availability of other promising biomarkers, such as cerebrospinal fluid (CSF) indices of Aß, tau and other pathologies.[117] Large-scale clinical studies should continuously be carried out to ascertain the comparative diagnostic accuracy and cost-benefit of novel PET and SPECT imaging probes and CSF markers, as well as the usefulness of employing such measurements in combination.

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** Modest*
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ARTIGO

Novos alvos moleculares para tomografia por emissão de pósitrons (PET) e tomografia computadorizada por emissão de fóton único (SPECT) em doenças neurodegenerativas

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DESCRITORES
Tomografia por emissão de pósitrons;
Tomografia computadorizada por emissão de fóton único;
Doenças neurodegenerativas;
Neuroinflamação;
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Resumo
A fisiopatologia das doenças neurodegenerativas (DN), tais como a doença de Alzheimer (DA) e a doença de Parkinson (DP), ainda não é completamente compreendida. No entanto, nos últimos anos, houve grandes avanços em termos do conhecimento sobre proteínas intra e extracelulares, tais como beta-amiloide (Aß), α-sinucleína, proteína tau e marcadores neuroinflamatórios, que podem ter sua função ou expressão prejudicada na DA, DP ou em outras DN. Progressos recentes nas técnicas de tomografia por emissão de pósitrons (PET) e tomografia computadorizada por emissão de fóton único (SPECT) permitem hoje em dia a identificação não invasiva de tais alvos moleculares *in vivo*. Este artigo resume descobertas recentes de estudos de PET e SPECT cerebral usando esses alvos moleculares inovadores e discute o papel potencial dessas técnicas no campo do desenvolvimento de novos medicamentos para as DN, bem como futuras aplicações clínicas em relação ao diagnóstico diferencial e monitoramento da progressão dessas doenças.

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Introdução

A tomografia por emissão de pósitrons (PET) e a tomografia computadorizada por emissão de fóton único (SPECT) são técnicas de imagem *in vivo* que permitem a identificação não invasiva de processos fisiopatológicos do cérebro subjacentes a diversos transtornos psiquiátricos e neurológicos. Essas técnicas também foram usadas com sucesso em vários aspectos do desenvolvimento das drogas, incluindo a compreensão do mecanismo de ação de agentes farmacológicos no sistema nervoso central (SNC), os regimes de dose e os limiares para a resposta clínica e o surgimento de efeitos colaterais.[1]

Nas últimas décadas, vários estudos mostraram que os métodos de PET e SPECT podem mapear com segurança processos neuroquímicos de interesse no cérebro, incluindo a densidade e a afinidade dos receptores pós-sinápticos como dopamina, serotonina e outros; de receptores pré-sinápticos desses neurotransmissores; de precursores como a L-DOPA e de enzimas que degradam os neurotransmissores. Tal abordagem tem fornecido informações bastante úteis sobre as anormalidades neuroquímicas envolvidas nos transtornos psiquiátricos e neurológicos, assim como auxiliado a elucidar o mecanismo de ação dos agentes farmacológicos comumente usados para tratar essas doenças.

Mais recentemente, os avanços tecnológicos possibilitaram o uso das técnicas de PET e SPECT para investigar uma série de outras proteínas intra e extracelulares que possam mostrar funções ou expressões prejudicadas nas doenças cerebrais. Tais avanços transformaram o uso neurológico e psiquiátrico de PET e SPECT: de obtenção de imagem com função estritamente neuroquímica para um perfil de aplicações muito mais flexível e abrangente, que proporciona conhecimento sobre os mecanismos cerebrais moleculares que possam se aproximar da essência fisiopatológica dos transtornos neurológicos e psiquiátricos do que as transformações superficiais de neurotransmissores.

Uma das mais promissoras aplicações dos novíssimos métodos de PET e SPECT se refere à investigação de aspectos fisiopatológicos de doenças neurodegenerativas (DN). Isso é de extrema importância, dada a grande prevalência de DN, tais como a doença de Alzheimer (DA) e a doença de Parkinson (DP), na terceira idade, bem como o fato de um conhecimento maior acerca da fisiopatologia dessas doenças poder auxiliar o desenvolvimento de novos tratamentos farmacológicos capazes de interferir no substrato molecular dessas patologias. Levando essas questões em consideração, esta revisão vai focar nas perspectivas para novos traçadores de PET e SPECT, desenvolvidos para permitir o mapeamento de mecanismos intra e extracelulares de particular relevância para DA, DP e outras DN.

Imagem molecular do cérebro com pet e spect: princípios básicos

Para permitir uma visualização e caracterização eficientes, além de medidas quantitativas de processos biológicos relevantes no cérebro, as técnicas de PET e SPECT demandam o desenvolvimento de sondas que podem ser marcadas com um isótopo emissor de pósitron (no caso de PET) ou isótopo emissor de fóton (no caso de SPECT). É importante ressaltar que, por causa da resolução espacial limitada, é normalmente necessário o uso de tomografia computadorizada (TC) ou de imagem por ressonância magnética (IRM). Com o uso de *software* especial, as técnicas funcionais e estruturais podem ser facilmente mescladas por meio da criação de imagens paramétricas. No entanto, o desenvolvimento de sistemas híbridos em que as técnicas funcionais estão completamente integradas aos métodos estruturais da estrutura também ajudou a atenuar a falta de resolução anatômica de PET e SPECT. Essas imagens paramétricas fornecem informações anatômicas e funcionais, permitindo a identificação de regiões que exibem diferenças na captação de compostos marcados. De qualquer forma, os radioisótopos mais empregados para se marcar as sondas de PET são carbono-11 (^{11}C) e flúor-18 (^{18}F), diferindo basicamente quanto à meia-vida e energia máxima. O primeiro (^{11}C) deve ser produzido por um cíclotron local situado próximo ao equipamento de imagem de PET por causa da meia-vida física muito curta (20 minutos). No entanto, a meia-vida mais longa de ^{18}F (110 minutos) permite a entrega de ligantes marcados com ^{18}F para uma lista maior de equipamentos de PET localizados na mesma cidade ou, até mesmo, em cidades vizinhas. Para imagens de SPECT, as sondas podem ser marcadas com iodo-123 (^{123}I) ou tecnécio-99m (^{99m}Tc);[2] esses isótopos possuem meia-vida muito mais longa do que os usados na imagem de PET e evitam a necessidade de um cíclotron próximo.

Tendo atravessado a barreira hematoencefálica (BBB) após a injeção intravenosa, o composto marcado por radionuclídeos se acumula em determinadas partes do cérebro, dependendo do processo biológico investigado. Tanto a PET quanto a SPECT são equipadas com detectores distintos de radiação colocados próximos à cabeça, após a injeção de radioligantes. Os dados coletados pelos detectores são transformados para gerar mapas tomográficos tridimensionais que mostram a distribuição regional da radioatividade emitida pelo cérebro. De modo a ser adequado para a imagem do cérebro *in vivo* com PET ou SPECT, um composto radiofarmacêutico (também chamado de radiotraçador, devido à dose subfarmacológica) precisa ser capaz de se ligar especificamente ao seu alvo (potencial de ligação de uma droga) (Figura 1); caso contrário, a precisão da informação da imagem obtida pode ser prejudicada. Por definição, o potencial de ligação (PL) é uma medida fundamental no uso da PET para mensurar a densidade dos receptores "disponíveis"; por exemplo, para avaliar a ocupação por drogas ou para caracterizar as anormalidades na distribuição do receptor em associação com os transtornos neuropsiquiátricos. Assim, PL é uma medida combinada que depende da densidade do receptor, bem como da afinidade do receptor à droga.[3]

Traçadores de imagem de amiloide

Placas senis extracelulares são agregados de proteína formados devido à falta de equilíbrio entre a produção e a liberação de proteínas ou de peptídeos no tecido cerebral de pacientes DA. O beta-amiloide (Aß), formado pela clivagem da proteína precursora de amiloide (PPA), é o constituinte mais importante presente nessas placas e representa a principal indicação caracterizadora do diagnóstico neuropatológico de DA. A clivagem de PPA pode ser feita por diversas proteases ou proteínas peptidases. Dentre elas, as secretases, especialmente gama (que contém presenilina, nicastrina,

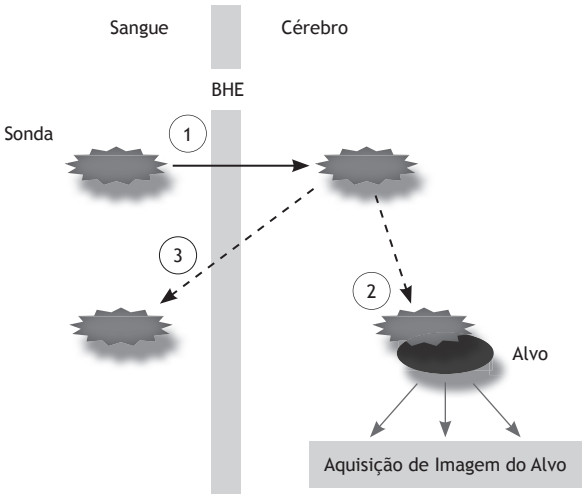

Figura 1 Os requisitos básicos para os agentes apropriados da imagem-alvo incluem: (1) pronto cruzamento da barreira hematoencefálica; (2) ligação seletiva às moléculas-alvo; e (3) sinais claros e contrastantes entre as moléculas-alvo e não alvo.[4]

anterior pharynx defective-1 e *presenilin enhancer-20*) e beta (*ß-site APP cleaving enzyme 1*, BACE1), são as enzimas mais importantes, sendo a atividade desempenhada por elas responsável pela liberação excessiva de peptídeos (Aß42) variantes de 42 aminoácidos altamente amiloidogênicos. Por outro lado, a clivagem de PPA promovida pelas secretases tipo α disintegrina e metaloproteinase (ADAM) 10 e 17 contribui para a formação de fragmentos solúveis de neuroproteção conhecidos como Sα-APP.[5]

O grande avanço do conhecimento sobre a base molécula da DA, descrita acima, gerou enorme interesse no desenvolvimento de traçadores de PET e SPECT que poderiam ser úteis para a imagem *in vivo* de placas Aß no cérebro humano. O primeiro traçador de PET desenvolvido para se ligar especificamente às placas fibrilares Aß foi o composto-B de Pittsburgh com marcação *11*C ([^{11}C]PIB). Até agora, esse foi o traçador de PET mais bem caracterizado e mais amplamente usado para o estudo de depósitos de amiloides no cérebro humano, tanto em DA quanto em outras DNs. Os vários possíveis papéis das técnicas de imagem com amiloide *in vivo* em DA estão resumidos na Tabela 1.

Como um diagnóstico definitivo de DA só pode ser confirmado por meio do exame neuropatológico *post-mortem*, ferramentas de diagnóstico que podem ser usadas para dar suporte a uma suspeita de DA em um indivíduo com déficit de memória e outras características de declínio cognitivo são altamente valiosas. Diversos estudos mostraram um grau marcado de retenção de [^{11}C]PIB no córtex de associação de pacientes com DA branda, se comparado a controles saudáveis[6-9] (Figura 2). Essas descobertas estabeleceram as imagens PET com [^{11}C]PIB como um recurso útil de aquisição de imagens para auxiliar a confirmação de diagnóstico de DA precoce.[9,10] No entanto, deve-se ressaltar que o depósito de amiloide não é patognômico da DA, sendo, por exemplo, encontrado em uma proporção de idosos cognitivamente saudáveis. No entanto, um resultado negativo de [^{11}C]PIB PET fornece informações importantes para descartar o diagnóstico de DA.

A utilidade da imagem de [^{11}C]PIB com PET para avaliar a evolução de DA também não foi estabelecida. Por exemplo, um interessante estudo de acompanhamento de pacientes com DA com duração de dois anos revelou que não houve aumento significativo na captação de [^{11}C]PIB ao longo do tempo, embora, individualmente, alguns pacientes tenham demonstrado um claro aumento.[8] Esse tipo de padrão de resultados indica um período de estabilidade do depósito de Aß quando já existe demência clínica. As investigações que usam PET com [^{11}C]PIB também são clinicamente úteis para auxiliar na distinção entre DA e outras demências. O mais notável é que pacientes com demência frontotemporal (DFT), em geral, apresentam uma captação normal de [^{11}C]PIB (embora pacientes com DFT ocasional possam apresentar uma captação maior no cérebro).[11,12]

Os indivíduos com declínio cognitivo objetivo, e não grave o suficiente para atender aos critérios de demência, recebem o diagnóstico de comprometimento cognitivo leve (CCL).[13] Indivíduos com o diagnóstico de CCL têm um alto risco de desenvolver demência, com uma taxa de conversão para DA estimada em aproximadamente 12% ao ano.[13] Vários estudos mostraram que uma subpopulação de indivíduos com CCL apresentou níveis mais altos de captação de [^{11}C]PIB no mesmo patamar do que foi observado em pacientes com DA.[7,14,15] Além disso, investigações recentes demonstram que a maior captação de [^{11}C]PIB no cérebro de pacientes com CCL é altamente preditiva da conversão subsequente de DA.[16]

Também é importante observar que a maioria dos pacientes com demência com corpos de Lewy (DCL) demonstra uma maior captação de [^{11}C]PIB no cérebro.[17] Relatórios recentes demonstraram que [^{11}C]PIB promete ajudar na distinção entre pacientes com DCL e pacientes com DP, DP com demência (DPD), DP com comprometimento cognitivo leve (DP-CCL) e indivíduos controles saudáveis (SCS).[17,18] No entanto, a retenção de [^{11}C]PIB não foi diferente entre os diagnósticos de DPD, DP-CCL, DP e SCS.[18] É importante observar que um estudo relatou que o aumento da retenção de [^{11}C]PIB no cérebro de pacientes com DCL é bastante atribuído à ligação de [^{11}C]PIB às placas Aß, e não à α-sinucleína, o componente estrutural primário das fibrilas dos corpos de Lewy.[19]

Finalmente, duas outras áreas importantes para o uso em potencial de traçadores de imagem de amiloide incluem o desenvolvimento de drogas e o monitoramento dos efeitos de tratamento (Tabela 1). Por exemplo, em um estudo com pacientes com DA tratados com fenserina, um composto anticolinesterásico, eles apresentaram uma melhora cognitiva. No entanto, esta não foi acompanhada por mudanças significativas na retenção cortical média de [^{11}C]PIB no cérebro.[20]

Dada a meia-vida mais longa de sondas marcadas com ^{18}F em comparação a compostos marcados com [^{11}C] (110 minutos *vs*. 20 minutos), nos últimos anos tem havido grande interesse pelo desenvolvimento de compostos marcados com ^{18}F para a imagem do amiloide cerebral com PET, que seria transportado de aparelhos de produção radiofarmacêutica a outros locais de imagem com PET. Alguns traçadores de imagem do amiloide, incluindo o [^{18}F]flutemetamol (um derivado de PIB marcado com ^{18}F), [^{18}F]AV-45 (florbetapir) e [^{18}F]BAY 94-9172 (florbetaben), já estão em estágio avançado em ensaios clínicos.[21-24] É importante frisar que o florbetapir foi recentemente aprovado pela *Food and Drug Administration* (FDA) nos Estados Unidos, para a obtenção de imagem

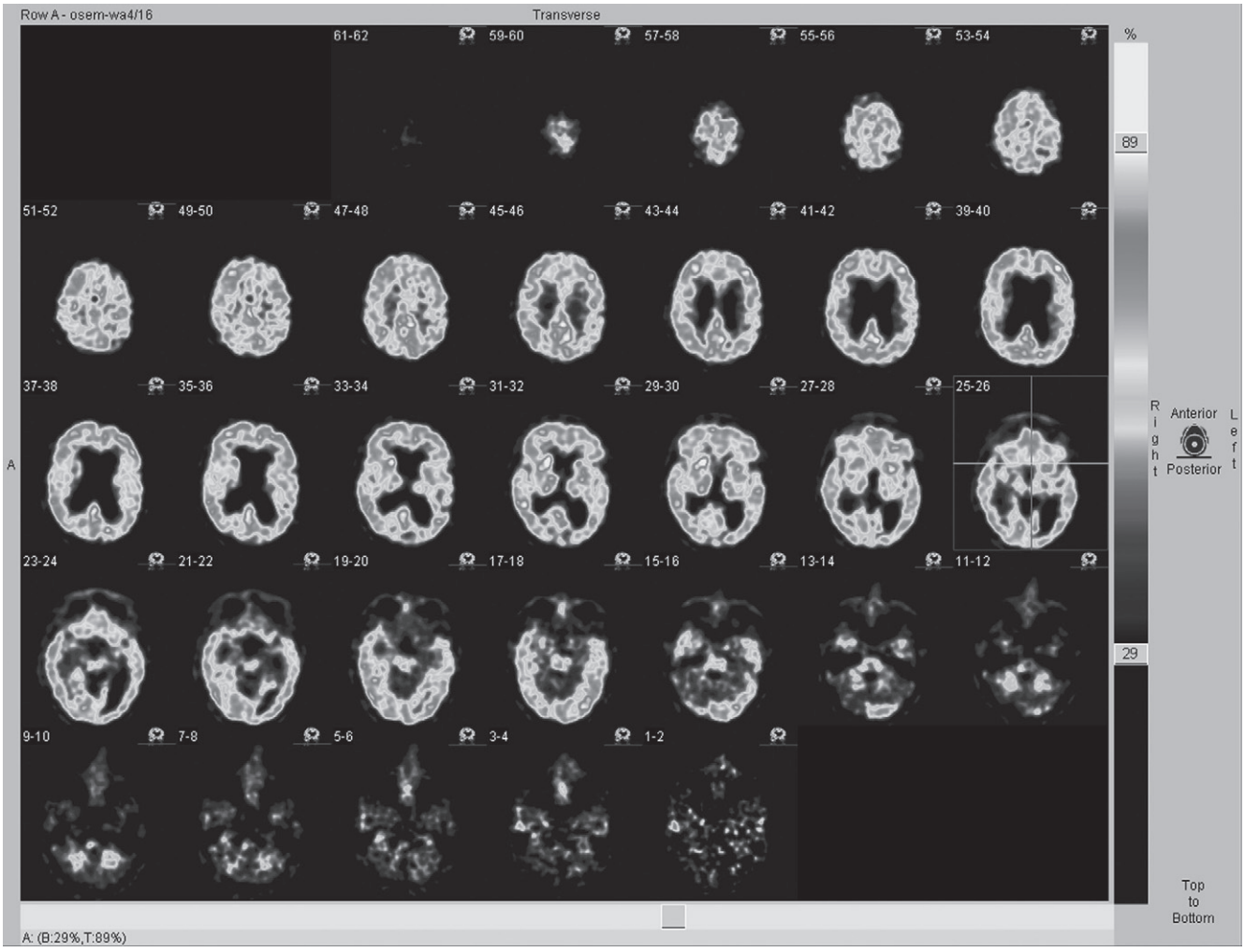

Figura 2 Imagens de PET obtidas após injeção intravenosa do composto B de Pittsburgh marcado com 11C (PiB) em um paciente com provável doença de Alzheimer que revelam a deposição de amiloide no cérebro. Cores mais quentes (e.g. vermelho e amarelo) indicam concentrações maiores de depósitos de amiloide, enquanto a cor azul indica a ausência de depósitos.

Tabela 1 Usos da imagem de amiloide com PET em transtornos neurodegenerativos

Aplicações da pesquisa
• Elucidação de aspectos patofisiológicos da doença de Alzheimer (DA), pequeno dano cognitivo e outros transtornos envolvendo a deposição de amiloide no cérebro
• Mapeamento da evolução das mudanças patológicas no cérebro ao longo do tempo
• Avaliação das propriedades modificadoras da doença em tratamentos novos
Possíveis aplicações clínicas
• Descarte da doença de Alzheimer em casos de suspeita de declínio cognitivo
• Diagnósticos diferenciados entre doença de Alzheimer e demência frontotemporal
• Diagnósticos diferenciados entre demência com corpos de Lewy e doença de Parkinson

cerebral com PET em adultos sob avaliação de DA e outras causas de declínio cognitivo.[25] Estudos clínicos indicam que esses traçadores de imagem do amiloide marcados com ^{18}F diferenciam bem pacientes com DA de sujeitos saudáveis. No entanto, esses estudos ainda apresentam limitações técnicas, incluindo um grau relativamente baixo de ligação específica *in vivo*, bem como um nível elevado de ligação de substância branca em cérebros de indivíduos saudáveis, o que reduz o contraste entre captação específica cortical e não cortical do traçador.

Além disso, dois ligantes marcados com tecnécio (^{99m}Tc) e rênio (Re) têm sido sintetizados recentemente para a imagem Aß com SPECT. Eles são derivados dos compostos (2-(1-(6-(*dialkylamino*)*naphthalen*-2-yl)e*thylidene*)*malononitrile* (DDNP) e 1-(6-(*dialkylamino*)*naphthalen*-2-yl) *ethanone* (ENE). No entanto, esses compostos mostraram pouca afinidade com os agregados de Aß e requerem maior refinamento para melhorar a difusão por meio da BBB.[26] Ademais, também é importante mencionar que a SPECT tem sensibilidade e resolução espacial mais baixas, se comparada ao método PET, e isso pode promover possíveis diferenças de precisão entre essas duas técnicas.

Traçadores de imagem de TAU

Além das placas Aß extracelulares, mencionadas acima, os emaranhados neurofibrilares intracelulares (NFTs), compostos de filamentos da proteína tau hiperfosforilada com ligação a microtúbulos, também são um marco importante para os transtornos neurodegenerativos, incluindo a DA, sendo preferencialmente localizados no hipocampo e nas regiões corticais associativas.[27,28] Pesquisas neuropatológicas anteriores sugerem que o depósito de NFTs ocorre antes da manifestação de sintomas clínicos em DA e é suficiente para fornecer um diagnóstico neuropatológico de DA.[29-31] Assim, a obtenção de imagem de NFTs *in vivo* juntamente com a de placas Aß seria útil para o diagnóstico precoce e preciso de DA. Uma avaliação quantitativa da patologia da tau também pode ser útil para se investigar a gravidade da demência, uma vez que o grau de depósito de NFTs se correlaciona bem com a gravidade clínica da demência. Por fim, como algumas formas de degeneração lobar frontotemporal são caracterizadas pelo acúmulo patológico da proteína tau, os traçadores de imagem de tau se mostram promissores para o diagnóstico de tais doenças epara a diferenciação entre elas, se comparadas à DA e aos transtornos psiquiátricos.[32]

O primeiro radiotraçador desenvolvido para a imagem da proteína tau no cérebro com PET foi o ^{18}F-FDDNP,[33,34] seguido do ^{18}F-FSB[35] e do ^{18}F-FP-curcumin.[36] No entanto, todos esses radioligantes se ligam tanto a NFTs quanto às placas Aß no cérebro.[36,36,37] Dessa forma, os traçadores apresentam valor limitado para investigar com precisão os aspectos de DA relacionados a tau ou reforçar o diagnóstico de DFT. Uma limitação adicional de [^{18}F]FDDNP são suas relações baixas de sinal/ruído para a imagem de PET, devido ao seu sinal de ligação específico reduzido e à rápida captação de metabólitos lipofílicos.[37,38]

No entanto, foi identificada, recentemente, uma série de derivados de quinolona que se ligam a NFTs de tau com maior afinidade do que as fibrilas de ß amiloide.[39] Um desses derivados, 2-(4-*aminophenyl*)-6-(2-([^{18}F]*fluoroethoxy*)) *quinolone* ([^{18}F]THK523), foi avaliado para a realização de imagens com PET da patologia da tau no cérebro.[40] O derivado demonstrou alta afinidade e seletividade para com as fibrilas de tau *in vitro* e *in vivo*. É interessante observar que esse traçador apresentou pouca ligação no cérebro de ratos transgênicos que expressam de maneira excessiva PPA com acúmulo significativo de Aß cerebral, demonstrando, assim, sua seletividade para a tau.[40] Além disso, as análises autorradiográficas e histoflorescentes das seções seriadas do hipocampo humano de pacientes com DA exibiram ligação THK523 positiva que se colocalizou na patologia de tau imunorreativa, não destacando placas Aß. Esses experimentos indicam que [^{18}F]THK523 atende aos critérios de um radioligante apropriado que poderia ser usado em ensaios com realização de imagem em humanos.

Mais recentemente, estudos *in vitro* e *in vivo* também mostraram que TH2, um novo derivado de rodanina e tioidantoina (TH) radioionizadas, se liga especificamente a NFTs e pode ser apropriado para a imagem com SPECT da patologia da tau.[41] Um outro traçador de SPECT em potencial é o derivado do fenildiazenil benzotiazol (PDB) 4-[2-(5-metoxi-2--benzotiazolil-diazenil]-N,N-dimetil-benzenamina, que se liga aos agregados de tau com uma seletividade dupla em relação aos agregados de Aß.[42] No entanto, experimentos de biodistribuição com o uso de ratos normais mostram que os derivados de BFD exibem níveis persistentes de radioatividade no cérebro. Isso faz com que atualmente sejam inapropriados para a imagem de NFTs *in vivo* em humanos. Talvez sejam necessárias mudanças estruturais no arranjo de PDB, de modo a tornar esses compostos úteis para a imagem de NFTs no cérebro humano com SPECT.

Traçadores de corpos de Lewy

Outra área de pesquisa importante se refere ao desenvolvimento de radiotraçadores empregados em PET e SPECT capazes de se ligar especificamente aos corpos de Lewy. Tais compostos podem ser muito úteis para o diagnóstico e a avaliação da terapia e o grau da progressão patológica de transtornos associados à α-sinucleína. A α-sinucleína é o constituinte principal dos corpos de Lewy e sabe-se que interage com várias proteínas envolvidas na neurodegeneração.[43] O acúmulo patológico pode alterar a função mitocondrial,[44] o rearranjo sináptico,[45] a proteína associada ao microtúbulo como a função de tau (porque pode interagir com a tubulina),[46,47] o comportamento neuronal do complexo de Golgi e o tráfego de vesículas[48] e a composição lipídica e fluidez da membrana celular.[49]

Recentemente, demonstrou-se que o composto BF227, em princípio planejado como um agente de imagem de Aß,[50] marca tanto as placas Aß quanto os corpos de Lewy em análises imunohistoquímicas/fluorescentes das seções dos cérebros daqueles que sofrem de DA e DP, respectivamente. Assim, [^{18}F]BF227 é considerado um: biomarcador não seletivo potencial de Aß para o estudo de DP.[51] Vale ressaltar que recentemente mostrou-se que BF227 mancha inclusões citoplasmáticas da glia contendo α-sinucleína em tecido *post-mortem*. No mesmo trabalho, os exames de PET com BF227 marcado com carbono ([^{11}C]BF227) detectaram depósitos de α-sinucleína no cérebro de pacientes com atrofia de múltiplos sistemas (AMS).[52] Isso indica que [^{11}C]BF227 poderia ser uma possível ferramenta para monitorar a eficácia da terapia de neuroproteção para α-sinucleinopatias. Todavia, estudos adicionais são necessários, de modo a verificar se os corpos de Lewy em outras α-sinucleinopatias, bem como inclusões citoplasmáticas da glia, podem ser detectados por [^{11}C]-BF-227 usando PET.

Traçadores de neuroinflamação

A neuroinflamação é conhecida como um processo multifatorial relacionado à idade, comumente encontrada em estágios precoces de DN, e está diretamente implicada na evolução dessas doenças.[53] Até agora, o traçador mais usado para visualizar a neuroinflamação no cérebro é o [^{11}C]PK11195, capaz de mapear a ativação micróglial através da ligação da proteína translocadora 18-kDa (TSPO), anteriormente conhecida como receptor periférico de benzodiazepínico (PBR). TSPO é basicamente encontrada na membrana mitocondrial externa e está, em primeiro lugar, envolvida no transporte de colesterol para a futura esteroidogênese. No tecido cerebral, a expressão de TSPO é relativamente baixa. No entanto, ocorre um significativo *up-regulation* quando a micróglia está ativada, o que confere a essa proteína um

papel importante como um biomarcador neuroinflamatório no cérebro.[54] Recentemente, [11C]PK11195 foi usado em vários estudos sobre transtornos psiquiátricos e revelou padrões de vasta ativação micróglial no cérebro (Figura 3). Em DN, estudos com traçadores mostraram que a ativação micróglial é de fato um evento patológico precoce,[55-58] dando, assim, suporte para o possível uso de intervenções terapêuticas baseadas em anti-inflamatórios para DNs. No entanto, é importante considerar que é também nos estágios precoces de DN que a micróglia possui efeitos de proteção, por exemplo, promovendo a eliminação do amiloide. Todavia, essas células se tornam cada vez mais disfuncionais em estágios posteriores, contribuindo para a evolução da doença.[59]

Infelizmente, questões cruciais limitaram o uso de [11C] PK11195 para medir a neuroinflamação no cérebro, incluindo a pobre biodisponibilidade no tecido cerebral e os altos níveis de ligação não específica.[60] O nível elevado de atividade não específica torna a interpretação de imagens muito complexa e confusa. Mais recentemente, vários outros traçadores PET relacionados a TSPO foram caracterizados e estão sendo usados em estudos pré-clínicos e clínicos sobre neuroinflamação em associação com DNs (veja Ching *et al.*,[61] para revisão). Tais radiotraçadores, incluindo [11C]DPA-713 e o derivado de 2-*Phenylimidazo*[1,2-*a*]*pyridineacetamide*, [11C]CLINME, também podem ser mais apropriados para a visualização da neuroinflamação branda do que [11C]-(*R*)-PK11195, dado que são mais sensíveis para a detecção de pequenas quantidades de TSPO; têm níveis mais baixos de ligação não específica; e fornecem razões mais altas de sinal-ruído. Tais propriedades foram avaliadas com o uso de modelos de infecção, pelos quais a taxa da captação do traçador nas áreas infectadas é comparada à captação em tecidos saudáveis.[62,63] Tais propriedades superiores em comparação a [11C]-(*R*)-PK11195 também foram demonstradas para [18F]PBR111, a versão fluorada de [11C]CLINME,[64] e [18F]DPA-714,[62] com a vantagem de que os últimos traçadores são marcados com 18F (meia-vida de 110 minutos). É importante ressaltar que estudos pré-clínicos recentes com imagem de TSPO foram conduzidos com sucesso usando modelos de esclerose múltipla e glioma com [18F]DPA-714.[65,66,67]

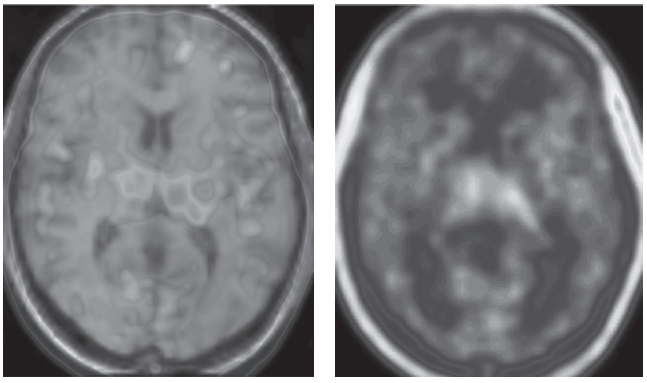

Figura 3 Imagem paramétrica de PET representativa de potencial de ligação (PL) de [11C]PK11195 em indivíduos saudáveis, sobrepostos em um template de IRM (imagem de ressonância magnética) (A); e uma imagem de PET [11C] PK11195 de um paciente com esquizofrenia (B). Repare na captação do radiotraçador no indivíduo com esquizofrenia (em azul), o que sugere a ocorrência de processos neuroinflamatórios.

Recentemente, surgiram alguns outros possíveis alvos moleculares da neuroinflamação. Foram feitas com sucesso imagens da atividade da ß-glucuronidase, uma enzima lisossômica liberada pelos astrócitos reativos e pela micróglia, em um modelo de rato com encefalite induzida por HSV-1 usando 18F-FEAnGA[68] (Figura 4). Ainda, mostrou-se o Local de Ligação de Imidazolina2 (I_2BS), I(2)R, está alterado em diversos transtornos cerebrais, incluindo a DA.[69] Alguns ligantes para I_2BS, incluindo o deprenil, são capazes de inibir a monoamina oxidase (MAO),[70] cuja atividade é elevada na astrocitose do cérebro humano em pacientes com DA (ou astrogliose, i.e., um aumento anormal do número de astrócitos devido à neurotoxicidade ou aos danos cerebrais), medidos por [11C]DED[71] (veja abaixo). Assim, a realização da imagem de I_2BS é uma ferramenta promissora para o estudo da neuroinflamação em DN. Recentemente, o ligante [11C]FTIMD foi avaliado com uma atividade aperfeiçoada e extremamente específica que detectou pequenas alterações na expressão de I(2)R no cérebro de ratos.[72] Outro ligante potencialmente útil é o BU99008, que demonstra melhor captação do cérebro *in vivo* e especificidades em comparação a [11C]FTIMD.[73]

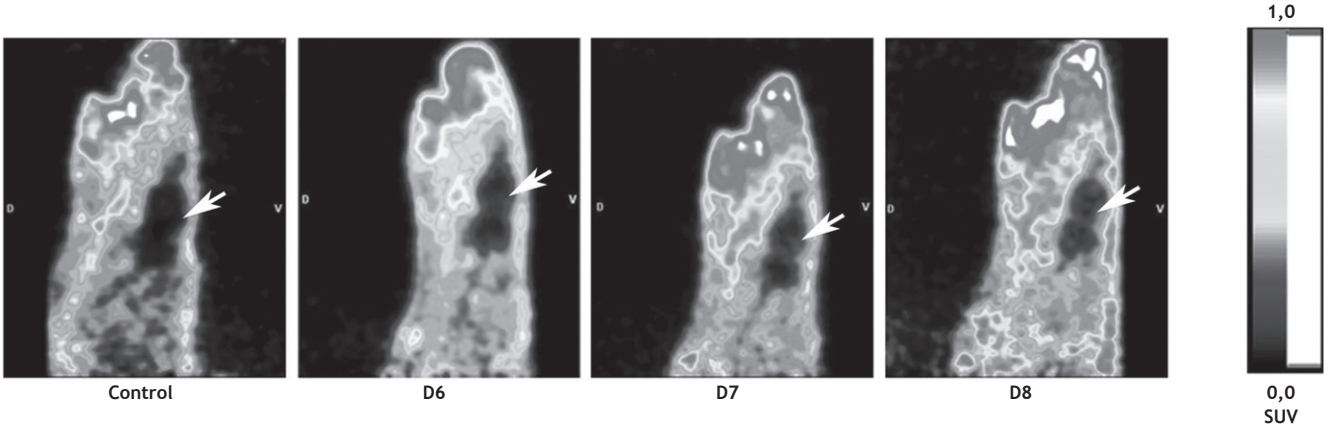

Figura 4 Visão sagital da cabeça de um rato controle (controle) e de um rato infectado com HSV-1 (dia 6 [D6]; dia 7 [D7] e dia 8 [D8] após a inoculação do vírus). As imagens representam a captação do traçador entre 10 e 60 minutos após a injeção de [18F]FEAnGA. Repare a ativação microglial dependente do tempo no cérebro (setas).

Por fim, as enzimas ciclo-oxigenase (COX) são amplamente conhecidas como um alvo molecular fundamental para drogas anti-inflamatórias. Recentemente, [^{11}C]*ketoprofen methyl ester* foi pré-clinicamente avaliado e comprovou-se que é eficiente na quantificação da expressão de COX-1 nos modelos de neuroinflamação e neurodegeneração. Além disso, gerou resultados melhores do que [^{11}C]PK11195 na quantificação da neuroinflamação. Sendo assim, [^{11}C]cetoprofeno metil éster demonstrou ser sensível a processos anti-inflamatórios com alvo em COX-1 na micróglia e nos macrófagos ativados.[74]

Além disso, como a astrocitose é um fenômeno comumente observado que está envolvido na neuroinflamação e neurodegeneração, isso também pode ser avaliado no cérebro humano usando traçadores PET. O radioligante mais essencial até agora avaliado para esse propósito é o ^{11}C-*deuterium*-L-*deprenyl* ou [^{11}C]DED, conforme mencionado anteriormente. Estudos recentes demonstraram um aumento na ligação de [^{11}C]DED por todo o cérebro de pacientes com DA que também apresentam níveis altos de captação de [^{11}C]PIB, sugerindo que a astrocitose é um fenômeno precoce no desenvolvimento de DA, provavelmente sendo uma intermediária entre a amiloidose e a perda neuronal.[75,76]

Por fim, também há resultados promissores no uso de traçadores SPECT para a neuroinflamação. Por exemplo, [^{123}I]PK11195 foi recentemente usado em um estudo piloto de SPECT com pacientes com DA.[77] Além disso, o composto 6-*chloro*-2-(4'*iodophenyl*)-3-(N,N-*diethyl*)-*imidazo*[1,2-a]*pyridine*-3- *acetamide* ou [^{123}I]CLINDE radiomarcado com ^{123}I também foi testado com sucesso em estudos pré-clínicos. Usando modelos animais diferentes de neuroinflamação, [^{123}I]CLINDE demonstrou bom desempenho para avaliar as mudanças de TSPO relacionadas tanto à ativação astroglial quanto à micróglial.[78,79] Há também investigações preliminares de [^{125}I]DPA-713 em ratos expostos a compostos neurotóxicos que induzem convulsões; esses estudos revelaram uma maior radioatividade do cérebro em ratos tratados com compostos neurotóxicos se comparados aos controles, que foram completamente bloqueados pela administração de PK11195.[80]

Traçadores do metabolismo lipídico do cérebro

O ácido araquidônico (AA) e o ácido docosahexaenoico (DHA), ácidos graxos poli-insaturados (AGP) da série ômega-6 e ômega-3, respectivamente, são constituintes importantes de fosfolipídios nas membranas celulares e contribuem extensamente para a sinalização celular no cérebro. AA se origina a partir da conversão do ácido linoleico, obtido por meio de dieta, enquanto que a concentração de DHA no cérebro depende do conteúdo da dieta de DHA, bem como da síntese pelo fígado de seu precursor, o ácido α-linolênico.[81,82] A resposta do SNC ao dano e ao início (e evolução) da neurodegeneração envolve a liberação de DHA e AA livres, juntamente com a síntese de derivados de docosanoides estereoespecíficos e prostanoides, respectivamente.[82,83] A liberação de AA em tais condições é mediada por fosfolipases específicas, por exemplo, PLA2, que contribuem para a conversão de AA em moléculas anti-inflamatórias, tais como a prostaglandina E2 (PGE2) pelas enzimas ciclo-oxigenases (COX) 1 e 2. É interessante observar que, na tentativa de compreender o papel de PLA2 em DN, um estudo recente demonstrou que a inibição de PLA2 no cérebro dos ratos leva a um decréscimo no total de proteína tau.[84] Por outro lado, DHA tem propriedades anti-inflamatórias e seu derivado de docosanoide (e.g. neuroprotectina D1) apresenta bioatividade de neuroproteção no cérebro contra diversos insultos, incluindo o dano oxidativo, a isquemia-reperfusão e a inflamação. Além disso, foram detectadas baixas concentrações no cérebro de pacientes com transtornos cerebrais, tais como DA e depressão.[83,85,86,87] É importante ressaltar que taxas medidas de AA e da incorporação de DHA aos fosfolipídios cerebrais representam as respectivas taxas de consumo metabólico, já que esses AGPs não são sintetizados de novo ou convertidos significativamente de seus precursores no cérebro[82] (veja abaixo).

Em recentes investigações sobre PET, foi observado um aumento de 26% na incorporação global de AA no cérebro de pacientes com DA, em comparação a indivíduos saudáveis, usando [^{11}C]ácido araquidônico ([^{11}C]AA).[88] Tal incorporação foi particularmente maior nas regiões do cérebro em que se imagina que a neuroinflamação esteja presente na DA e [^{11}C]AA poderia, portanto, ser um marcador novo de micróglia ativada a ser usado em estudos com transtornos neurodegenerativos. Outros estudos avaliaram o traçador [1-(11)C]DHA marcado com pósitron para mapear a incorporação do DHA plasmático não esterificado no cérebro de adultos voluntários saudáveis. Valores de coeficientes k* de incorporação para DHA foram mais altos nas regiões cerebrais com substância cinzenta do que com substância branca. Para o cérebro humano inteiro, a taxa total de incorporação de DHA, J_{in} – o produto de k* e da concentração plasmática de DHA não esterificado – foi equivalente a 3,8 ± 1,7 mg/dia. Os autores destacaram que essa taxa total, que se aproxima da taxa total do consumo de DHA pelo cérebro, é menor do que a quantidade diária de 200 mg de suplementação de DHA recomendada.[89] Sendo assim, com o uso de [1-(11)C]DHA, é possível quantificar o metabolismo regional e global de DHA em seres humanos quanto à saúde e doença.[90] Além disso, um estudo mais recente demonstrou que é possível medir a incorporação de DHA plasmático *in vivo* pelo cérebro. Dessa forma, a imagem quantitativa da incorporação de DHA do plasma para o cérebro pode ser usada como um biomarcador *in vivo* do metabolismo de DHA no cérebro e da neurotransmissão.[87] É importante destacar que isso pode auxiliar a monitorar o consumo de DHA *in vivo* em pacientes com transtornos como depressão e DA, em que a suplementação de DHA pode ser útil.[91-93]

Outros novos alvos moleculares para neuroimagem em neurodegeneração

Um potente e seletivo inibidor da proteína quinase C (PQC), a Enzastaurina (LY317615), foi recentemente marcado com ^{11}C para aplicações de imagem com PET ([^{11}C]Enzastaurina).[94] PQC é uma enzima envolvida em alguns mecanismos biológicos da célula e é um dos mais importantes elementos iniciais que atuam na indução das já mencionadas α-secretases, a ADAM-10 e 17, que fazem parte da neuroproteção.[95] Ainda, recentemente sintetizou-se a sonda sensível à mielina, [^{11}C]MeDAS, e provou-se que ela

é eficaz na detecção de mudanças de mielina no cérebro. Esse radiotraçador, que pode ser usado como um marcador de imagem da mielina para monitorar a degeneração de mielina *in vivo*,[96] é um avanço potencialmente útil para a investigação de DN, já que a degeneração de neurônios, axônios e sinapses está presente de forma clara na DA, bem como na esclerose múltipla.[97]

As proteínas de choque térmico (HSP) também exercem papéis importantes na neuroproteção. HSP70, por exemplo, é uma proteína conhecida, encontrada em agressomas de corpos de Lewy, que está basicamente implicada na degradação de proteínas aberrantes.[98] O agressoma é uma resposta geral das células que ocorre quando a capacidade do proteassoma (envolvido na degradação de proteínas não usuais) é excedida pela produção de proteínas com enovelamento não adequado, propícias à agregação.[99] De modo similar, as catepsinas também são fundamentais para a degradação de enzimas que podem estar implicadas em DN.[100] Sendo assim, a função prejudicada dessas proteínas pode facilitar a evolução de DN. É interessante observar que o sistema de repórteres de PET (i.e., que usa genes repórteres) para obter a imagem da expressão de genes no cérebro intacto foi recentemente usado para gerar a imagem e monitorar a ativação do fator de choque térmico 1 (HSF1)/ fator de transcrição HSP70.[101] Além disso, recentemente, outro grupo produziu a imagem da atividade da catepsina cisteína com o uso de ^{64}Cu-Z-FK(DOTA)-AOMK e PET. A aquisição de imagens dessas proteínas com o uso do traçador radioisotópico ^{18}F mais amplamente disponível pode constituir um recurso excelente para o diagnóstico precoce e para o monitoramento da evolução da doença de DN.

Respostas imunes inatas também desempenham um papel importante na neurodegerenação. Por exemplo, foi descoberto recentemente que os monócitos e a micróglia, que são deficientes para o fator 88 de diferenciação mieloide (MyD88) (envolvido na via de sinalização do receptor do tipo Toll), exibem uma reação fagocitária funcionalmente deficiente para Aβ.[102] Além disso, MyD88 está envolvido na degeneração neuronal dopaminérgica induzida pela neurotoxina MPTP no sistema nervoso entérico (SNE) do rato.[103] No entanto, essa neurodegeneração não é um mecanismo dependente de MyD88.[104] Assim, é necessário maior conhecimento antes de se considerar estudos com imagens de PET/SPECT para essa nova proteína-alvo.

O estresse oxidativo (EO) que ocasiona dano mitocondrial é um fenômeno importante e precoce que aciona a neurodegeneração.[105,106] É interessante observar que um traçador denominado [^{62}Cu]ATSM, inicialmente delineado para o estudo da hipóxia tumoral,[107] foi recentemente usado, pela primeira vez, para avaliar o EO em DP. Esse estudo demonstrou que o EO estriado se intensificou em pacientes com DP, se comparado a controles, e aumentou com a evolução da severidade da doença, particularmente no estriado contralateral.[108] Também foi demonstrado que esse traçador é muito específico para as células com disfunção mitocondrial, até mesmo sob normóxia, o que sugere, portanto, que [^{62}Cu]ATSM pode ser realmente um traçador interessante para o estudo de transtornos cerebrais que envolvem a disfunção mitocondrial, tais como DA e DP.[109]

A glicoproteína-P (P-gp) é um transportador da BBB de efluxo ativo envolvido na neuroproteção. Sua disfunção é considerada uma das causas do início de DP[110,111] e DA.[112] Além disso, também foi estabelecida *in vivo* uma correlação entre idade e um decréscimo da função desse transportador.[113] Sendo assim, desenvolver métodos para obter imagens de P-gp em tais doenças é um desafio fundamental atualmente. Diversos estudos já foram conduzidos com o uso do substrato [^{11}C]verapamil radiomarcado com P-gp nos estudos com PET. No entanto, metabólitos polares radiomarcados são formados após a injeção de radioligantes e isso pode resultar no sinal de P-gp não mediado como um fator de confusão.[114] Logo, são necessários novos traçadores de P-gp para obter imagens da função P-gP.

Considerações finais

Os estudos revisados neste artigo demonstram as várias oportunidades a serem exploradas usando os já existentes traçadores de imagens moleculares disponíveis que mapeiam os alvos relevantes para DN, incluindo Aβ, α-sinucleína, proteína tau e marcadores neuroinflamatórios.

Por outro lado, os mecanismos do cérebro subjacentes às DN ainda não foram totalmente elucidados e outros alvos de possível relevância para as DN surgem continuamente.[115] É evidente a necessidade de desenvolver e usar novos compostos de aquisição de imagens moleculares para alvos desse tipo, além dos relacionados a Aβ, α-sinucleína e proteína tau, de modo a adquirir um conhecimento mais completo da base molecular de DA, DP e outras DN. Espera-se que com o uso dos novos compostos de aquisição de imagens moleculares, os métodos PET e SPECT poderão auxiliar a compreender melhor os processos patológicos subjacentes e as alterações moleculares específicas que surgem durante os estágios precoces de DN.

Com um número cada vez maior de unidades de pesquisa animal no mundo e com acesso à tecnologia micro-PET e SPECT para estudos pré-clínicos, espera-se que estudos farmacológicos que usam novos radiotraçadores possam auxiliar na identificação e validação dos processos moleculares como biomarcadores novos a serem usados como alvos terapêuticos para tratamento, serem como na avaliação da maneira pela qual novas drogas são capazes de modificar esses biomarcadores nos modelos animais de DN. Nesse campo, uma das estratégias mais promissoras deveria ser o uso de protocolos com multitraçadores para a avaliação simultânea de diferentes alvos moleculares de relevância para a DN. Por exemplo, investigações recentes com o uso de ratos transgênicos que expressam patologias que caracterizam tanto a demência com corpos de Lewy (DCL) quanto a DA (DCL-DA rato) revelaram que Aβ, tau e α-sinucleína agem sinergicamente para promover a agregação, a fosforilação e o acúmulo de cada uma, bem como levam ao acelerado declínio cognitivo.[116] Assim, os protocolos com multitraçadores para esses três alvos moleculares devem ser fortemente considerados em investigações de DN.

Finalmente, para os próximos anos, seria bastante recomendado traduzir algumas das novas descobertas descritas acima para aplicações diagnósticas na prática clínica. Com tais desenvolvimentos, os padrões de imagem de PET e SPECT podem ser usados mais incisivamente para aperfeiçoar a precisão do diagnóstico em casos de dúvida, bem como prever prognósticos e respostas ao tratamento em pacientes com

DN. A maior disponibilidade do SPECT e o baixo custo podem torná-lo o método escolhido para futuras aplicações clínicas, porém espera-se também acesso crescente aos métodos de PET em um número maior de hospitais, particularmente quanto ao uso de traçadores marcados com ^{18}F. O uso de qualquer radiotraçador novo para aplicações clínicas com PET ou SPECT deveria ser comparado à disponibilidade de outros biomarcadores promissores, como as dosagens de AB [símbolos alfa beta], tau e outras no líquido cefalorraquidiano (LCR).[117] Estudos clínicos em larga escala deveriam ser conduzidos continuamente, de modo a assegurar a precisão diagnóstica comparativa e o custo-benefício de novas sondas de imagem PET e SPECT e marcadores LCR, bem como a utilidade de se empregar em conjunto tais medições.

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